Table 1 Mean Animal Use[1] for the UDP[2] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with Various Regressions – All Chemicals

Assay/Regression	With Default Starting Dose[3]	With NRU-Based Starting Dose[4]	Animals Saved[5]	With Default Starting Dose[3]	With NRU-Based Starting Dose[5]	Animals Saved[5]	Accuracy[6]
3T3 NRU Test Method	**Dose-Response Slope = 2**			**Dose-Response Slope = 8.3**			
RC millimole[6]	8.62 ± 0.18	8.08 ± 0.22	0.53 (6.2%)	7.06 ± 0.21	6.52 ± 0.25	0.54 (7.7%)	26%
RC rat-only weight[7]	9.61± 0.17	8.86 ± 0.18	0.75* (7.8%)	8.19 ± 0.19	7.49 ± 0.22	0.70* (8.6%)	35%
RC rat-only weight excluding substances with specific mechanisms of toxicity[8]	9.61 ± 0.17	8.91 ± 0.19	0.69* (7.2%)	8.21 ± 0.19	7.54 ± 0.24	0.67* (8.2%)	46%
NHK NRU Test Method	**Dose-Response Slope = 2**			**Dose-Response Slope = 8.3**			
RC millimole[6]	8.56 ± 0.18	8.15 ± 0.22	0.42 (4.9%)	7.01 ± 0.20	6.58 ± 0.25	0.43 (6.1%)	28%
RC rat-only weight[7]	9.60± 0.17	8.94 ± 0.18	0.66* (6.9%)	8.19 ± 0.19	7.57 ± 0.23	0.62* (7.6%)	30%
RC rat-only weight excluding substances with specific mechanisms of toxicity[8]	9.59 ± 0.16	8.93 ± 0.20	0.66* (6.9%)	8.20 ± 0.19	7.56 ± 0.25	0.64* (7.8%)	38%

[1]Numbers are mean numbers of animals and standard errors for 2000 simulations for each of 70 substances for the 3T3 NRU test method and 71 substances for the NHK NRU test method. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. The slight differences in the number of animals used for the default starting dose at the same dose-response slope reflect different simulation runs.
[2]OECD (2001a); EPA (2002a).
[3]Default starting dose = 175 mg/kg.
[4]Starting dose = one default dose lower than the NRU-predicted LD_{50} calculated using the geometric mean of the laboratory geometric mean NRU IC_{50} values in the specified regression.
[5]Difference between mean animal use with default starting dose and mean animal use with NRU-based LD_{50}. Differences denoted by * were statistically significant (i.e., $p < 0.05$) by a one-sided Wilcoxon signed rank test. Percentage difference is shown in parentheses.
[6]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Tables 6-4** to **6-6**).
[7]$\log LD_{50}$ (mmol/kg) = 0.435 $\log IC_{50}$ (mM) + 0.625.
[8]$\log LD_{50}$ (mg/kg) = 0.372 $\log IC_{50}$ (μg/mL) + 2.024.
[9]$\log LD_{50}$ (mg/kg) = 0.357 $\log IC_{50}$ (μg/mL) + 2.194.

Table 2 Animal Use[1] for the UDP[2] by GHS Toxicity Category[3] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with the RC Millimole Regression[4] – All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			
Toxicity Category[3]	**Number of Reference Substances**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**Accuracy[8]**
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	12	10.67 ± 0.24	9.62 ± 0.37	1.05* (9.9%)	9.42 ± 0.20	8.64 ±0.34	0.78 (8.3%)	0%
$5 < LD_{50} \leq 50$ mg/kg	12	8.98 ± 0.34	8.85 ± 0.50	0.13 (1.5%)	7.63 ± 0.31	7.46 ± 0.37	0.17 (2.2%)	17%
$50 < LD_{50} \leq 300$ mg/kg	12	7.78 ± 0.11	8.33 ± 0.24	-0.55 (-7.0%)	6.67 ± 0.23	6.97 ± 0.25	-0.30 (-4.5%)	67%
$300 < LD_{50} \leq 2000$ mg/kg	12	9.05 ± 0.22	8.84 ± 0.16	0.21 (2.3%)	7.77 ± 0.27	7.55 ± 0.24	0.22 (2.8%)	100%
$2000 < LD_{50} \leq 5000$ mg/kg	11	8.66 ± 0.28	7.37 ± 0.40	1.29* (14.9%)	5.64 ± 0.40	4.32 ± 0.56	1.32* (23.4%)	0%
$LD_{50} > 5000$ mg/kg	11	6.47 ± 0.32	5.23 ± 0.47	1.24* (19.2%)	5.02 ± 0.02	3.81 ± 0.31	1.21* (24.1%)	10%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	12	10.51 ± 0.24	10.23 ± 0.35	0.27 (2.6%)	9.39 ± 0.20	9.11 ± 0.35	0.28 (2.9%)	0
$5 < LD_{50} \leq 50$ mg/kg	12	9.06 ± 0.34	8.76 ± 0.47	0.30 (3.3%)	7.63 ± 0.30	7.37 ± 0.33	0.25 (3.3%)	50%
$50 < LD_{50} \leq 300$ mg/kg	12	7.80 ± 0.12	8.19 ± 0.25	-0.39 (-5.0%)	6.61 ± 0.22	6.87 ± 0.23	-0.26 (-4.0%)	50%
$300 < LD_{50} \leq 2000$ mg/kg	12	9.04 ± 0.22	8.73 ± 0.15	0.32 (3.5%)	7.73 ± 0.27	7.47 ± 0.23	0.26 (3.4%)	100%
$2000 < LD_{50} \leq 5000$ mg/kg	11	8.65 ± 0.29	7.55 ± 0.34	1.11* (12.8%)	5.64 ± 0.40	4.51 ± 0.46	1.13* (20.0%)	9%
$LD_{50} > 5000$ mg/kg	12	6.42 ± 0.29	5.41 ± 0.48	1.01* (15.7%)	5.02 ± 0.02	3.99 ± 0.29	1.02* (20.4%)	0%

[1]Numbers are mean numbers of animals used and standard errors for 2000 simulations for each substance with a limit dose of 5000 mg/kg. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the initial LD_{50} values from **Table 3-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. The slight differences in the number of animals used for the default starting dose at the same dose-response slope reflect different simulation runs.

[2]OECD (2001a); EPA (2002a).

[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).

[4]RC millimole regression is log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625.

[5]Default starting dose = 175 mg/kg.

[6]Starting dose was one default dose lower than the predicted LD_{50} calculated using the geometric mean of the laboratory geometric mean NRU IC_{50} values in the RC millimole regression.

[7]Difference between mean animal use with default starting dose and mean animal use with NRU predicted LD_{50}. Differences marked by * are statistically significant ($p < 0.05$) by a one-sided Wilcoxon signed rank test. Percentage difference shown in parentheses

[8]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Table 6-4**).

Table 3 Animal Use[1] for the UDP[2] by GHS Toxicity Category[3] Using Starting Doses Based on the NRU Test Methods with the RC Rat-Only Weight Regression[4]-All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			Accuracy[8]
Toxicity Category[3]	Number of Reference Substances	With Default Starting Dose[5]	With NRU-Based Starting Dose	Animals Saved[7]	With Default Starting Dose[5]	With NRU-Based Starting Dose	Animals Saved[7]	
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	11.50 ± 0.15	11.18 ± 0.38	0.32 (2.8%)	10.37 ± 0.29	10.11 ± 0.41	0.27 (2.6%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	9.51 ± 0.21	9.01 ± 0.34	0.50 (5.3%)	8.55 ± 0.23	8.02 ± 0.34	0.52 (6.1%)	17%
$> 50 < LD_{50} \leq 300$ mg/kg	12	7.73 ± 0.12	7.99 ± 0.21	-0.26 (-3.4%)	6.83 ± 0.21	7.04 ± 0.27	-0.22 (-3.2%)	67%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	8.74 ± 0.25	7.97 ± 0.10	0.77* (8.8%)	7.61 ± 0.30	7.00 ± 0.21	0.61 (8.0%)	100%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	10.79 ± 0.08	8.97 ± 0.29	1.82* (16.8%)	9.17 ± 0.25	7.47 ± 0.42	1.69* (18.5%)	0%
> 5000 mg/kg	12	9.72 ± 0.28	8.17 ± 0.44	1.55* (16.0%)	6.82 ± 0.36	5.26 ± 0.51	1.56* (22.9%)	10%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	11.40 ± 0.18	11.66 ± 0.25	-0.25 (-2.2%)	10.34 ± 0.30	10.58 ± 0.32	-0.24 (-2.3%)	0
$> 5 < LD_{50} \leq 50$ mg/kg	12	9.68 ± 0.21	8.55 ± 0.28	1.13* (11.7%)	8.59 ± 0.23	7.68 ± 0.27	0.92* (10.7%)	50%
$> 50 < LD_{50} \leq 300$ mg/kg	12	7.80 ± 0.12	8.13 ± 0.17	-0.32 (-4.1%)	6.96 ± 0.22	7.24 ± 0.28	-0.28 (-4.1%)	50%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	8.75 ± 0.25	8.01 ± 0.11	0.75* (8.5%)	7.61 ± 0.30	6.98 ± 0.21	0.63 (8.3%)	100%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	10.73 ± 0.07	9.12 ± 0.22	1.60*(14.9%)	9.17 ± 0.26	7.65 ± 0.37	1.52* (16.6%)	9%
$LD_{50} > 5000$ mg/kg	13	9.56 ± 0.28	8.25 ± 0.42	1.31*(13.7%)	6.76 ± 0.34	5.37 ± 0.47	1.39* (20.6%)	0%

[1]Numbers are mean number of animals used and standard errors for 2000 simulations for each substance with a limit dose of 5000 mg/kg. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the reference LD_{50} values from **Table 4-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. The slight differences in the number of animals used for the default starting dose at the same dose-response slope reflect different simulation runs.

[2]OECD (2001a); EPA (2002a).

[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).

[4]From **Table 6-2**; $\log LD_{50}$ (mg/kg) = 0.372 $\log IC_{50}$ (μg/mL) + 2.024

[5]Default starting dose = 175 mg/kg.

[6]Starting dose was one default dose lower than NRU-predicted LD_{50} calculated using the geometric mean of the laboratory geometric mean NRU IC_{50} values in the RC rat-only regression.

[7]Difference between mean animal use with default starting dose and mean animal use with NRU predicted LD_{50}. Differences marked by * were statistically significant (i.e., $p < 0.05$) by a one-sided Wilcoxon signed rank test. Percent difference is shown in parentheses.

[8]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Table 6-5**).

Table 4 Animal Use[1] for the UDP[2] By GHS Toxicity Category[3] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with the RC Rat-Only Weight Regression Excluding Substances with Specific Mechanisms of Toxicity[4]-All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			
Toxicity Category[3]	**Number of Reference Substances**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose**	**Animals Saved[7]**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose**	**Animals Saved[7]**	**Accuracy[8]**
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	11.41 ± 0.16	11.58 ± 0.35	-0.16 (-1.4%)	10.33 ± 0.29	10.52 ± 0.39	-0.19 (-1.9%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	9.42 ± 0.20	9.29 ± 0.35	0.13 (1.3%)	8.51 ± 0.23	8.25 ± 0.34	0.26 (3.1%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	7.78 ± 0.10	8.27 ± 0.21	-0.49* (-6.3%)	6.94 ± 0.19	7.33 ± 0.25	-0.39 (-5.6%)	80%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	8.79 ± 0.26	7.86 ± 0.08	0.93* (10.6%)	7.62 ± 0.30	6.88 ± 0.18	0.74 (9.7%)	78%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	10.83 ± 0.07	8.67 ± 0.24	2.16* (19.9%)	9.17 ± 0.25	7.18 ± 0.37	1.99* (21.7%)	67%
> 5000 mg/kg	12	9.73 ± 0.28	7.93 ± 0.44	1.80* (18.5)%	6.82 ± 0.36	4.99 ± 0.52	1.83* (26.9%)	25%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	11.34 ± 0.19	11.95 ± 0.19	-0.61* (-5.4%)	10.33 ± 0.30	10.88 ± 0.26	-0.55* (-5.4%)	0
$> 5 < LD_{50} \leq 50$ mg/kg	12	9.60 ± 0.21	8.77 ± 0.32	0.83* (8.6%)	8.55 ± 0.23	7.85 ± 0.31	0.70 (8.2%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	7.81 ± 0.11	8.37 ± 0.23	-0.56* (-7.2%)	7.03 ± 0.21	7.48 ± 0.29	-0.45 (-6.4%)	60%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	8.80 ± 0.26	7.81 ± 0.09	0.99* (11.3%)	7.63 ± 0.31	6.79 ± 0.19	0.84* (11.0%)	89%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	10.76 ± 0.07	8.87 ± 0.25	1.89* (17.6%)	9.18 ± 0.26	7.41 ± 0.39	1.78* (19.4%)	44%
$LD_{50} > 5000$ mg/kg	13	9.56 ± 0.28	7.93 ± 0.45	1.62* (17.0%)	6.76 ± 0.34	5.05 ± 0.50	1.70* (25.2%)	15%

[1]Numbers are mean number of animals used and standard errors for 2000 simulations for each substance with a limit dose of 5000 mg/kg. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the reference LD_{50} values from **Table 4-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. The slight differences in the number of animals used for the default starting dose at the same dose-response slope reflect different simulation runs.
[2]OECD (2001a); EPA (2002a).
[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).
[4]From **Table 6-2**; log LD_{50} (mg/kg) = 0.357 log IC_{50} (μg/mL) + 2.194.
[5]Default starting dose = 175 mg/kg.
[6]Starting dose = One default dose lower than NRU-predicted LD_{50} calculated using the geometric mean of laboratory mean IC_{50} values in the RC rat-only weight regression excluding substances with specific mechanisms of toxicity.

Table 5 Animal Use[1] for the ATC[2] Using Starting Doses Based on NRU Test Methods with Various Regressions –All Chemicals

Assay/Regression	With Default Starting Dose[3]	With NRU-Based Starting Dose[4]	Animals Saved[5]	With Default Starting Dose[3]	With NRU-Based Starting Dose[5]	Animals Saved[5]	Accuracy[6]
3T3 NRU Test Method	**Dose-Response Slope = 2**			**Dose-Response Slope = 8.3**			
RC millimole[7]	10.79 ± 0.12	9.93 ± 0.24	0.86* (8.0%)	10.57 ± 0.17	9.73 ± 0.26	0.85* (8.0%)	26%
RC rat-only weight[8]	10.79 ± 0.12	9.53 ± 0.23	1.26* (11.7%)	10.57 ± 0.17	9.17 ± 0.26	1.40* (13.3%)	35%
RC rat-only weight excluding substances with specific mechanisms of toxicity[9]	10.79 ± 0.12	9.46 ± 0.22	1.33* (12.3%)	10.57 ± 0.17	9.01 ± 0.24	1.56* (14.8%)	46%
NHK NRU Test Method	**Dose-Response Slope = 2**			**Dose-Response Slope = 8.3**			
RC millimole[7]	10.82 ± 0.12	9.85 ± 0.24	0.97* (8.9%)	10.60 ± 0.17	9.62 ± 0.26	0.98* (9.2%)	28%
RC rat-only weight[8]	10.82 ± 0.12	9.67 ± 0.23	1.14* (10.6%)	10.60 ± 0.17	9.40 ± 0.25	1.20* (11.3%)	30%
RC rat-only weight excluding substances with specific mechanisms of toxicity[9]	10.82 ± 0.12	9.53 ± 0.20	1.29* (11.9%)	10.60 ± 0.17	9.17 ± 0.22	1.43* (13.5%)	38%

[1]Numbers are mean numbers of animals used and standard errors for 2000 ATC simulations each for 70 substances for the 3T3 NRU test method and 71 substances for the NHK NRU test method. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. Limit dose = 2000 mg/kg

[2]OECD (2001d).

[3]Default starting dose = 300 mg/kg.

[4]Starting dose was one fixed dose lower than NRU-predicted LD_{50} calculated using the geometric mean of laboratory mean IC_{50} values in the regression specified.

[5]Difference between mean animal use with default starting dose and mean animal use with NRU-based LD_{50}. Percentage difference is shown in parentheses. Differences marked by * were statistically significant (i.e., $p < 0.05$) using a one-sided Wilcoxon signed rank test.

[6]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Tables 6-4** to **6-6**).

[7]$\log LD_{50}$ (mmol/kg) = 0.435 $\log IC_{50}$ (mM) + 0.625.

[8]$\log LD_{50}$ (mg/kg) = 0.372 $\log IC_{50}$ (μg/mL) + 2.024.

[9]$\log LD_{50}$ (mg/kg) = 0.357 $\log IC_{50}$ (μg/mL) + 2.194.

Table 6 Animal Savings[1] for the ATC[2] by GHS Toxicity Category[3] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with the RC Millimole Regression[4] – All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			
Toxicity Category[3]	**Number of Reference Substances**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**Accuracy[8]**
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	12	9.51 ± 0.12	7.98 ± 0.68	1.63* (17.1%)	9.10 ± 0.07	7.44 ± 0.68	1.66* (18.3%)	0%
$5 < LD_{50} \leq 50$ mg/kg	12	11.95 ± 0.15	10.36 ± 0.71	1.58* (13.3%)	12.02 ± 0.07	10.38 ± 0.59	1.64* (13.7%)	17%
$50 < LD_{50} \leq 300$ mg/kg	12	10.76 ± 0.23	10.37 ± 0.20	0.39 (3.6%)	9.70 ± 0.32	9.53 ± 0.17	0.17 (1.8%)	67%
$300 < LD_{50} \leq 2000$ mg/kg	12	9.86 ± 0.09	10.10 ± 0.15	-0.24 (-2.5%)	9.37 ± 0.14	9.80 ± 0.26	-0.43* (-4.6%)	100%
$2000 < LD_{50} \leq 5000$ mg/kg	11	11.18 ± 0.08	11.02 ± 0.13	0.16 (1.4%)	11.90 ± 0.04	11.55 ± 0.20	0.35* (2.9%)	0%
$LD_{50} > 5000$ mg/kg	11	11.89 ± 0.03	9.97 ± 0.91	1.92 (16.2%)	12.00 ± 0.00	9.95 ± 0.97	2.05 (17.1%)	10%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	12	9.52 ± 0.12	8.50 ± 0.71	1.02 (10.7%)	9.10 ± 0.07	8.08 ± 0.66	1.02 (11.2%)	0%
$5 < LD_{50} \leq 50$ mg/kg	12	12.00 ± 0.15	9.09 ± 0.42	2.86* (23.9%)	12.02 ± 0.07	9.09 ± 0.19	2.93* (24.4%)	50%
$50 < LD_{50} \leq 300$ mg/kg	12	10.78 ± 0.24	10.55 ± 0.25	0.23 (2.1%)	9.71 ± 0.32	9.82 ± 0.41	-0.11 (-1.1%)	50%
$300 < LD_{50} \leq 2000$ mg/kg	12	9.86 ± 0.09	9.831 ± 0.13	0.02 (0.2%)	9.38 ± 0.14	9.43 ± 0.18	-0.06 (-0.6%)	100%
$2000 < LD_{50} \leq 5000$ mg/kg	11	11.19 ± 0.09	10.81 ± 0.27	0.38* (3.4%)	11.90 ± 0.04	11.17 ± 0.51	0.73 (6.2%)	9%
$LD_{50} > 5000$ mg/kg	12	11.91 ± 0.02	10.22 ± 1.01	1.69 (14.2%)	12.00 ± 0.00	10.19 ± 1.01	1.81 (15.1%)	0%

[1]Numbers are mean number of animals used and standard errors for 2000 simulations for each substance with a limit dose of 2000 mg/kg. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the initial LD_{50} values from **Table 3-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. Although the simulations used whole animals, averaging the results produced fractional numbers of animals.
[2]OECD (2001d).
[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).
[4]RC millimole regression is $\log LD_{50}$ (mmol/kg) = 0.435 $\log IC_{50}$ (mM) + 0.625.
[5]Default starting dose = 300 mg/kg.
[6]Starting dose was the next fixed dose lower than the predicted LD_{50} from using the NRU IC_{50} in the RC millimole regression.
[7]Difference between mean animal use with default starting dose and mean animal use with NRU-based starting dose. Statistically significant differences (i.e., $p < 0.05$) by a one-sided Wilcoxon signed rank test are noted by *. Percentage difference is shown in parentheses.
[8]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Table 6-4**).

.

Table 7 Animal Savings[1] for the ATC[2] by GHS Toxicity Category[3] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with the RC Rat-Only Weight Regression[4] – All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			Accuracy[8]
Toxicity Category[3]	Number of Reference Substances	With Default Starting Dose[5]	With NRU-Based Starting Dose[6]	Animals Saved[7]	With Default Starting Dose[5]	With NRU-Based Starting Dose[6]	Animals Saved[7]	
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	9.51 ± 0.12	7.94 ± 0.63	1.58* (16.6%)	9.10 ± 0.07	7.50 ± 0.62	1.60* (17.6%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	11.95 ± 0.15	9.97 ± 0.54	1.98* (16.6%)	12.02 ± 0.07	9.97 ± 0.41	2.04* (17.0%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	10.76 ± 0.23	10.30 ± 0.19	0.47 (4.3%)	9.70 ± 0.32	9.44 ± 0.23	0.27 (2.8%)	80%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	9.86 ± 0.09	10.14 ± 0.20	-0.28 (-2.8%)	9.37 ± 0.14	9.89 ± 0.36	-0.52 (-5.6%)	78%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	11.18 ± 0.08	9.79 ± 0.47	1.39* (12.4%)	11.90 ± 0.04	9.34 ± 0.82	2.56* (21.5%)	44%
> 5000 mg/kg	12	11.89 ± 0.03	9.01 ± 0.93	2.88* (24.2%)	12.00 ± 0.00	8.93 ± 0.99	3.07* (25.6%)	0%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	9.52 ± 0.12	8.29 ± 0.61	1.23* (13.0%)	9.10 ± 0.07	7.86 ± 0.56	1.23* (13.6%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	11.95 ± 0.15	9.26 ± 0.43	2.69* (22.5%)	12.02 ± 0.07	9.28 ± 0.29	2.74* (22.8%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	10.78 ± 0.24	10.51 ± 0.25	0.27 (2.5%)	9.71 ± 0.32	9.81 ± 0.42	-0.10 (-1.1%)	60%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	9.86 ± 0.09	9.95 ± 0.14	-0.10 (-1.0%)	9.38 ± 0.14	9.55 ± 0.24	-0.17 (-1.8%)	89%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	11.19 ± 0.09	10.45 ± 0.40	0.73 (6.6%)	11.90 ± 0.04	10.55 ± 0.69	1.35 (11.3%)	11%
$LD_{50} > 5000$ mg/kg	13	11.91 ± 0.02	9.47 ± 0.93	2.44 (20.5%)	12.00 ± 0.00	9.38 ± 0.98	2.62* (21.8%)	8%

[1]Numbers are mean number of animals used and standard errors for 2000 simulations for each substance with a limit dose of 2000 mg/kg. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the reference LD_{50} values from **Table 4-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU.
[2]OECD (2001d).
[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).
[4]From **Table 6-2;** $\log LD_{50}$ (mg/kg) = 0.372 $\log IC_{50}$ (μg/mL) + 2.024
[5]Default starting dose = 300 mg/kg.
[6]Starting dose was one fixed dose lower than the NRU-predicted LD_{50} calculated using the NRU IC_{50} in the RC rat-only weight regression.
[7]Difference between mean animal use with default starting dose and mean animal use with NRU-based LD_{50}. Differences marked by * were statistically significant (i.e., $p < 0.05$) by a one-sided Wilcoxon signed rank test. Percentage difference is shown in parentheses.
[8]Proportion of substances for which the GHS acute oral toxicity category (UN 2005) predicted by the *in vitro* NRU test methods matched the *in vivo* category (from **Table 6-5**).

Table 8 Animal Savings[1] for the ATC[2] By GHS Toxicity Category[3] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods with the RC Rat-Only Weight Regression Excluding Substances with Specific Mechanisms of Toxicity[4]- All Chemicals

		Dose-Response Slope = 2			Dose-Response Slope = 8.3			
Toxicity Category[3]	**Number of Reference Substances**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**With Default Starting Dose[5]**	**With NRU-Based Starting Dose[6]**	**Animals Saved[7]**	**Accuracy[8]**
		3T3 NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	9.51 ± 0.12	8.30 ± 0.61	1.22 (12.8%)	9.10 ± 0.07	7.85 ± 0.61	1.25 (13.7%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	11.95 ± 0.15	10.50 ± 0.54	1.45* (12.1%)	12.02 ± 0.07	10.51 ± 0.45	1.51* (12.6%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	10.76 ± 0.23	10.37 ± 0.22	0.39 (3.6%)	9.70 ± 0.32	9.48 ± 0.25	0.22 (2.3%)	80%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	9.86 ± 0.09	9.87 ± 0.10	-0.02 (-0.2%)	9.37 ± 0.14	9.45 ± 0.17	-0.08 (-0.9%)	78%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	11.18 ± 0.08	9.50 ± 0.47	1.67* (15.0%)	11.90 ± 0.04	8.83 ± 0.82	3.07* (25.8%)	67%
> 5000 mg/kg	12	11.89 ± 0.03	8.19 ± 0.78	3.70* (31.1%)	12.00 ± 0.00	8.02 ± 0.82	3.98* (33.1%)	25%
		NHK NRU Test Method						
$LD_{50} \leq 5$ mg/kg	7	9.52 ± 0.12	8.80 ± 0.45	0.72 (7.6%)	9.10 ± 0.07	8.38 ± 0.40	0.72 (7.9%)	0%
$> 5 < LD_{50} \leq 50$ mg/kg	12	11.95 ± 0.15	9.69 ± 0.53	2.26* (18.9%)	12.02 ± 0.07	9.70 ± 0.43	2.32* (19.3%)	14%
$> 50 < LD_{50} \leq 300$ mg/kg	12	10.78 ± 0.24	10.48 ± 0.23	0.30 (2.8%)	9.71 ± 0.32	9.74 ± 0.35	-0.03 (-0.3%)	60%
$> 300 < LD_{50} \leq 2000$ mg/kg	16	9.86 ± 0.09	9.78 ± 0.10	0.08 (0.8%)	9.38 ± 0.14	9.33 ± 0.14	0.04 (0.5%)	89%
$> 2000 < LD_{50} \leq 5000$ mg/kg	11	11.19 ± 0.09	9.96 ± 0.45	1.23* (11.0%)	11.90 ± 0.04	9.62 ± 0.80	2.28* (19.2%)	44%
$LD_{50} > 5000$ mg/kg	13	11.91 ± 0.02	8.44 ± 0.76	3.47* (29.2%)	12.00 ± 0.00	8.30 ± 0.81	3.70* (30.8%)	15%

[1]Numbers are mean number of animals used and standard errors for 2000 simulations for each substance with a limit dose of 2000 mg/kg. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Results are provided for 70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method categorized using the reference LD_{50} values from **Table 4-2**. No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU.
[2]OECD (2001d).
[3]GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD_{50} in mg/kg (UN 2005).
[4]From **Table 6-2**; log LD_{50} (mg/kg) = 0.357 log IC_{50} (μg/mL) + 2.194.
[5]Default starting dose = 300 mg/kg.
[6]Starting dose was one fixed dose lower than the NRU-predicted LD_{50} calculated using the NRU IC_{50} in the RC rat-only weight regression excluding substances with specific mechanisms of toxicity.
[7]Difference between mean animal use with default starting dose and mean animal use with NRU-based LD_{50}. Statistically significant differences (i.e., $p < 0.05$) by a one-sided Wilcoxon signed rank test are noted by *. Percentage difference is shown in parentheses.

Table 9 Animal Deaths[1] for the ATC[2] Using Starting Doses Based on the 3T3 and NHK NRU Test Methods – All Chemicals

Assay/ Regression	Default Starting Dose[3]			NRU-Based Starting Dose[4]		
	Used	Dead	% Deaths	Used	Dead	% Deaths
3T3 NRU	**Dose-Response Slope = 2**					
RC millimole[5]	10.79	4.13	38.3%	9.93	3.52	35.4%
RC rat-only[6]	10.79	4.13	38.3%	9.53	3.47	36.4%
RC rat-only excluding substances with specific mechanisms of toxicity[7]	10.79	4.13	38.3%	9.46	3.63	38.3%
	Dose-Response Slope = 8.3					
RC millimole[5]	10.57	3.67	34.7%	9.73	3.11	31.9%
RC rat-only[6]	10.57	3.67	34.7%	9.17	3.05	33.3%
RC rat-only excluding substances with specific mechanisms of toxicity[7]	10.57	3.67	34.7%	9.01	3.20	35.5%
NHK NRU	**Dose-Response Slope = 2**					
RC millimole[5]	10.82	4.07	37.7%	9.85	3.41	34.6%
RC rat-only[6]	10.82	4.07	37.7%	9.67	3.39	35.1%
RC rat-only excluding substances specific mechanisms of toxicity[7]	10.82	4.07	37.7%	9.53	3.55	37.2%
	Dose-Response Slope = 8.3					
RC millimole[5]	10.60	3.63	34.2%	9.62	2.99	31.1%
RC rat-only[6]	10.60	3.63	34.2%	9.40	2.98	31.7%
RC rat-only excluding substances with specific mechanisms of toxicity[7]	10.60	3.63	34.2%	9.17	3.13	34.1%

[1]Numbers are mean numbers of animals used for 2000 simulations for each substance (70 substances in the 3T3 NRU test method and 71 substances in the NHK NRU test method). No IC_{50} was obtained for carbon tetrachloride in either assay or for methanol in the 3T3 NRU. Although the simulations used whole animals, averaging the results produced fractional numbers of animals. Upper limit dose = 2000 mg/kg.
[2]OECD (2001d).
[3]Default starting dose = 300 mg/kg.
[4]Starting dose was one fixed dose lower than the NRU-predicted LD_{50}.
[5]log LD_{50} (mmol/kg) = 0.435 log IC_{50} (mM) + 0.625.
[6]log LD_{50} (mg/kg) = 0.372 log IC_{50} (μg/mL) + 2.024.
[7]log LD_{50} (mmol/kg) = 0.357 log IC_{50} (mM) + 2.194.

Unfortunately we do not have the numbers on animal deaths for the UDP at this time.